Effects of inducers of differentiation on protein kinase C and CMP-N-acetylneuraminic acid:lactosylceramide sialyltransferase activities of HL-60 leukemia cells

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Abstract Exposure of HL-60 leukemia cells to either 12-O-tetradecanoylphorbol-13-acetate (TPA), dimethylsulfoxide (DMSO), exogenous gangliosides GM3, GM1, or bovine brain ganglioside mixture (BBG) resulted in a marked inhibition of the growth of cells. The order of the inhibitory potency was TPA > GM3 > DMSO > BBG > GM1. In contrast, sulfatides were without effect on cellular replication. Treatment of HL-60 cells with TPA or GM3 induced differentiation along the monocyte/macrophage lineage, while treatment with DMSO induced maturation along the granulocytic pathway. These effects were accompanied by more than a twofold increase in protein kinase C (PKC) activity. In contrast, treatment with GM1, BBG, or sulfatides caused only a relatively small increase in PKC activity. The activity of CMP-N-acetylneuraminic acid:lactosylceramide sialyltransferase (ST1), a key enzyme for membrane gangliosides synthesis, in HL-60 cells was also influenced by the exposure to TPA, GM3, DMSO, GM1, or sulfatides. The inducers of differentiation, TPA and DMSO, caused an increase in ST1 activity, whereas GM3, which also induced cellular differentiation, inhibited ST1 activity, perhaps through the action of end-product inhibition. The non-inducers of differentiation, GM1 and sulfatides, also increased the activity of ST1, but to a much lesser extent. The findings suggest that the direct or indirect modulation of PKC activity by some of these agents may be involved, at least in part, in the regulation of cellular growth and differentiation. Furthermore, it is conceivable that differences in PKC activity may be responsible for the changes in ST1 activity associated with cell differentiation and proliferation. **—Xia, X-J., X-B. Gu, A. C. Sartorelli, and R. K. Yu.** Effects of inducers of differentiation on protein kinase C and CMP-N-acetylneuraminic acid:lactosylceramide sialyltransferase activities of HL-60 leukemia cells. *J. Lipid Res.* 1989. **30:** 181–188.

Supplementary key words gangliosides • protein kinase C • sialyltransferase • 12-O-tetra-decanoylphorbol-13-acetate • sulfatide • dimethylsulfoxide

The human promyelocytic cell line HL-60 can be induced to mature along either the monocyte/macrophage or granulocyte pathways by exposure to different chemical reagents. Thus, upon treatment with DMSO these cells differentiate to the granulocyte series (1), whereas exposure to TPA causes monocyte/macrophage maturation (2, 3). Recently, Nojiri et al. (4) reported that ganglioside GM3 also possessed the ability to induce the differentiation of HL-60 cells, as well as that of another human leukemia cell line, U937. The ganglioside GM3 is ubiquitous in that it is present in all well-differentiated cells, and it is commonly perceived as a marker for cellular differentiation. Thus, a characteristic increase in the level of GM3 was observed during monocyte/macrophage-like differentiation of many human myelocytic and monocytic leukemia cell lines, such as HL-60 (5), K562 (6), KG-1 (7), ML-1 (8), as well as HO melanoma cell differentiation (9), when these lines were treated with TPA. Furthermore, a corresponding increase in the activity of CMP-N-acetylneuraminic acid:lactosylceramide sialyltransferase (ST1) accompanied that of GM3 in HL-60 cells treated with TPA (10) and clonal hamster NIL 8-HSV cells (11). A net increase in GM3 biosyn-

Abbreviations: TPA, 12-O-tetradecanoylphorbol-13-acetate; DMSO, dimethylsulfoxide; Sulf, sulfatide; BBG, bovine brain ganglioside mixture (BBG contains four major gangliosides, GM1, GD1a, GD1b, and GT1b; together they account for nearly 95% of total gangliosides (45). The amount of GM3 present in the mixture was less than 1%); PKC, protein kinase C; PS, phosphatidylserine; NeuAc, N-acetylneuraminic acid; ST1, CMP-N-acetylneuraminic acid:lactosylceramide sialyltransferase; FBS, fetal bovine serum; NBT, nitroblue tetrazolium; TCA, trichloroacetic acid; PBS, phosphate-buffered saline; LacCer, lactosylceramide; PDGF, platelet-derived growth factor; EGF, epidermal growth factor; EGTA, ethylene glycol bis(β-aminoethyl ether)N,N'-tetraacetic acid; C, chloroform; M, methanol; W, water. The ganglioside nomenclature is that of Svennerholm (46): GM3, NeuAcα2 → 3Galβ1 → 4Glcβ1 → 1Cer; GM1, Galβ1 → 3GalNAcβ1 → 4[NeuAcα2 → 3]Galβ1 → 4Glcβ1 → 1Cer; GD1a, NeuAcα2 → 3Galβ1 → 3GalNAcβ1 → 4[NeuAcα2 → 3]Galβ1 → 4Glcβ1 → 1Cer; GD1b, Galβ1 → 3GalNAcβ1 → 4[NeuAcα2 → 8NeuAcα2 → 3]Galβ1 → 4Glcβ1 → 1Cer; GT1b, NeuAcα2 → 3Galβ1 → 3GalNAcβ1 → 4[NeuAcα2 → 8NeuAcα2 → 3]Galβ1 → 4Glcβ1 → 1Cer.

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thesis was also observed in TPA-treated HO melanoma cells using [^{14}C]glucosamine as a precursor (9).

Another important biochemical event associated with the action of TPA involves its effects on PKC. In HL-60 cells, the TPA receptor and PKC activity co-purify (12–14). TPA directly activates this kinase (15) and induces the phosphorylation of several proteins in HL-60 cells (16–20). In addition, cellular adherence, a measure of monocytic maturation, is blocked by palmitoylcarnitine, an inhibitor of PKC (21). These findings suggest a role for PKC in the induction of cell differentiation by TPA.

Since ST1 represents a key glycosyltransferase in the biosynthesis of gangliosides, it is possible that a relationship exists between the activities of PKC and ST1 during the maturation of HL-60 cells induced by TPA, DMSO, and GM3. In the present investigation, we have found that all three of these agents initiated cellular differentiation and enhanced PKC activity. TPA and DMSO also increased ST1 activity. The findings suggest that PKC activity may modulate the increment of ST1 activity, which may be of importance to the maturation of HL-60 cells regardless of whether they enter the granulocytic or monocytic/macrophagic pathways.

MATERIALS AND METHODS

Materials

DEAE-Sephadex A-25, Iatrobeads, and HPTLC plates were obtained from Pharmacia Fine Chemicals (Uppsala, Sweden), Iatron Industries, Inc. (Tokyo, Japan), and E. Merck Co. (Darmstadt, West Germany), respectively. DEAE-Sepharose CL-6B-100, Octyl-Sepharose CL-4B, Triton X-100, TPA, DMSO, Type III-S histone, and phosphatidylserine (PS) were purchased from Sigma Chemical Co. (St. Louis, MO). CMP-sialic acid ([4-^{14}C]sialic acid]) (1.8 mCi/mmol) was purchased from New England Nuclear Corp. (Boston, MA) and [γ-^{32}P]ATP (300 Ci/mmol) was from Amersham Corporation (Arlington Heights, IL). Gangliosides and sulfatides were isolated and purified from bovine or human brain by the method of Ledeen and Yu (22). Individual gangliosides and sulfatides were all demonstrated to be chromatographically pure on HPTLC plates. All other reagents were of at least analytical grade.

Cell culture

HL-60 leukemia cells were grown in RPMI 1640 medium (Gibco, Grand Island, New York) supplemented with 10% heat-inactivated fetal bovine serum (FBS, Gibco), 100 IU/ml of penicillin, and 100 μg/ml of streptomycin at 37°C, in a humidified atmosphere of 95% air–5% CO_2. Cells were used between passages 30 and 50 and were seeded at a level of 2×10^5 cells/ml in RPMI 1640 medium containing a reduced FBS concentration (5%) and either 10 nM TPA, 1.3% DMSO, 100 μM GM3, 100 μM GM1, 100 μM BBG, or 100 μM sulfatides.

Measurement of cellular proliferation and differentiation

Cell numbers were determined using a Coulter ZM particle counter. Macrophage-like cellular differentiation was induced by exposure of HL-60 cells to 10 nM TPA. After removal of nonadherent cells by washing with PBS, adherent cells were recovered by scraping with a rubber policeman. The number of adherent cells was expressed as a percentage of the total number of cells.

Granulocytic maturation of HL-60 leukemia cells was assessed by measurement of the reduction of NBT (23). Phagocytosis was measured by counting the number of cells containing two or more fluorescent microspheres (24). Briefly, about 1×10^6 cells in RPMI 1640 medium were centrifuged at 1,000 *g* for 5 min and the pellet was resuspended in 0.5 ml of medium containing 15% FBS. Fluorescent microspheres (Fluoresbrite carboxylate microspheres, diameter 1.09 μm, Polysciences, Inc., Warrington, PA) were sterilized with 70% ethanol for 1 hr, centrifuged, and resuspended in culture medium containing 15% FBS. The number of microspheres per ml, determined with a fluorescence microscope, was 4×10^7. To 0.5 ml of cell suspension was added 0.5 ml of the solution containing fluorescent microspheres. After 24 hr of incubation, the cells were separated from free microspheres by washing twice with PBS. The cells were fixed with 1% paraformaldehyde and stored at 4°C for 1 week before the number of cells containing microspheres was determined using a fluorescence microscope.

Extraction and purification of glycosphingolipids

After incubation for 3 days in the absence or presence of various agents, cells were harvested and washed with PBS at least 3 times. After the addition of 0.2 to 0.4 ml of H_2O, cell pellets were sonicated for 3 min and the protein concentration was determined by the method of Lowry et al. (25) using bovine serum albumin as the standard.

The sonicate, containing 1 mg of protein with 50 μg BBG added as a carrier, was resuspended in 6 ml of chloroform–methanol 1:1 (v/v). The mixture was sonicated for 10 min and the solvent was adjusted to chloroform–methanol–water 30:60:8 (v/v, solvent A) and further stirred overnight. After centrifugation, pellets were extracted with about 10 ml of solvent A and stirred overnight. The supernatants were combined and applied to a DEAE-Sephadex A-25 column (bed volume, 1.5 ml). The neutral glycolipid fraction was eluted with 15 ml of solvent A, and the acidic lipid fraction containing gangliosides, was eluted with 20 ml of chloroform–methanol–aqueous 0.8 M sodium acetate 30:60:8 (v/v).

Protein kinase C assay

After 3 days in culture in the presence or absence of various agents, the cells were harvested and washed twice with PBS. PKC was partialy purified as described by Kreutter, Caldwell, and Morin (26). Briefly, the cell pellet was resuspended in lysis buffer (20 mM Tris-HCl, 0.1 mM EGTA, 10% sucrose, 50 mM 2-mercaptoethanol, pH 7.5) and disrupted by sonication for 2 min. Triton X-100 was added to a final concentration of 0.3%. The mixture was incubated on ice for 15 min to solubilize membrane-bound PKC, and the resulting solution was applied to a small DEAE-cellulose column equilibrated in lysis buffer. The column was washed with lysis buffer and the enzyme was eluted with 0.1 M NaCl in lysis buffer. The enzyme was freshly prepared for each experiment. The activity of PKC was measured by the incorporation of ^{32}P from [γ-^{32}P]ATP into histone III-S. The reaction mixture for assaying PKC activity contained 10 mM Mg acetate, 1 mM EGTA, 1.1 μM Ca^{2+}, 0.01 mM ATP, 20 μg/ml of PS, 2 μg/ml of 1,2-diolein, 200 μg/ml of histone III-S, and 15 to 40 μg/ml of PKC. Reactions were carried out at 37°C for 15 min, and the reaction was terminated by the addition of 1.0 ml of 25% ice-cold trichloroacetic acid (TCA). After 5 min, the mixture was filtered (Millipore HA, 0.45 μm) and washed twice with 4 ml of 5% ice-cold TCA. The filters were placed in scintillation vials with 5 ml of Opti-Fluor scintillation fluid and ^{32}P incorporation into histone III-S was determined using an LKB Rackbeta scintillation counter.

CMP-N-acetylneuraminic acid:lactosylceramide sialyltransferase assay

After 3 days in culture in the presence of various agents, cells were harvested and washed twice with PBS. The cell pellets was resuspended in distilled water and disrupted by sonication for 2 min. The sonicate was used as the enzyme source. The reaction mixture for the ST1 assay contained 40 nmol of LacCer, 80 nmol of CMP-[^{14}C]NeuAc, 300 μg of Triton CF-54, 2 μmol of $MnCl_2$, 500 nmol of cacodylate buffer (pH 6.3), and 150 to 800 μg of protein in a final volume of 0.2 ml (27). The LacCer in chloroform–methanol 1:1 (v/v), Triton CF-54 in methanol, and CMP-[^{14}C]NeuAc in ethanol–water 1:1 (v/v) were dried in the incubation tube under N_2 prior to the addition of other reagents. The reaction was carried out at 37°C for 1 hr and was stopped by boiling the reaction mixture for 30 sec. Appropriate blanks were also processed with each assay which consisted of: *a*) boiled enzyme, and *b*) no LacCer, in order to determine the activity due to endogenous substrate in the enzyme preparation.

Isolation and assay of labeled gangliosides

Octyl-Sepharose CL-4B (0.5 ml) was added to each reaction mixture which was then incubated at 37°C for 1 hr. The suspension was applied to a small column containing 0.5 ml of Octyl-Sepharose CL-4B. The column was washed with 8 ml of distilled water and gangliosides (including unlabeled and labeled gangliosides) were eluted with 8.0 ml of chloroform–methanol 1:1 (v/v). The eluate was dried under a stream of N_2 and dissolved in 150 μl of chloroform–methanol 1:1 (v/v) for thin-layer chromatography.

HPTLC plates were developed in two different solvent systems, run consecutively in the same direction. The first solvent system was chloroform–methanol 85:15 (v/v), and the second was chloroform–methanol–water 55:45:10 (v/v) containing 0.02% $CaCl_2 \cdot 2H_2O$. Plates were exposed to Kodak X-ray film for 3 to 4 days to reveal radiolabeled gangliosides. Alternatively, plates were sprayed with resorcinol–HCl reagent to reveal gangliosides. The area corresponding to GM3 was removed by scraping the TLC plates and the silica gel was transferred to a scintillation vial containing 0.1 ml of water and 5 ml of Opti-Fluor. Vials were sonicated for 5 min and the radioactivity was determined using an LKB 1219 Rackbeta scintillation counter.

Protein assay

Protein concentrations were determined by the method of Lowry et al. (25) or a modified Coomassie blue R-250 binding protein assay using crystalline bovine serum albumin as the standard (28).

RESULTS

HL-60 leukemia cells were cultured in the presence of either TPA, DMSO, GM3, GM1, BBG, or sulfatides to determine the effects of these agents on cellular growth and differentiation. All of these agents, except the sulfatides, inhibited cellular replication, with the order of inhibitory potency being TPA > GM3 > DMSO > BBG > GM1 (**Fig. 1**). The growth rate of HL-60 cells was also found to decrease with increasing concentrations of GM1 or BBG in the culture medium.

The ability of mature monocytes and granulocytes to phagocytize particles is commonly used as a marker for myeloid cell differentiation. Cells incubated with TPA or DMSO differentiated into mature cells, with a marked increase in their capacity to express phagocytic activity (**Table 1**). Furthermore, the number of NBT-positive cells increased significantly in cells exposed to DMSO for 3 to 8 days, and the percentage of adherent cells increased in cells treated with TPA for 12 to 36 hr (Table 1). A minor increase in NBT-positivity was observed when cells were cultured with GM3, but not with GM1, BBG, or sulfatides (data not shown).

To determine the effects of the inducers of differentiation on PKC activity, enzyme activity was measured after cells were treated for 3 days, using partially purified en-

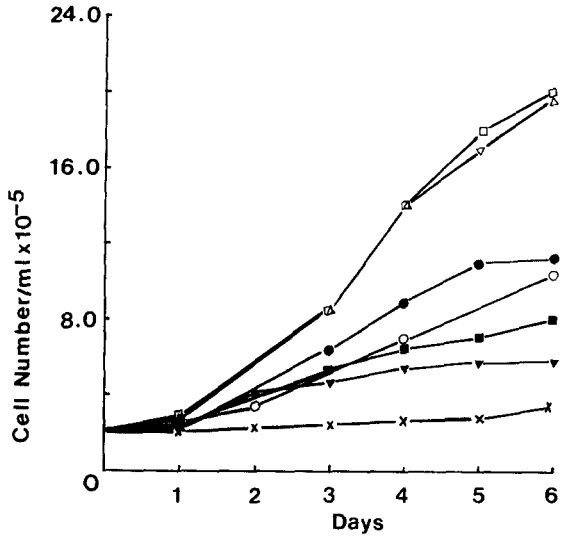

Fig. 1. Effects of exogenously added TPA, DMSO, gangliosides, and sulfatides on the growth of HL-60 leukemia cells. HL-60 cells were grown in RPMI 1640 medium containing 5% FBS in the absence of added agents (□) or in the presence of either 10 nM TPA, (X); 1.3% DMSO (■); 100 μM GM3, (▼); 100 μM GM1, (●); 100 μM BBG, (○); or 100 μM sulfatides, (△), and the number of cells was determined using a Coulter ZM particle counter. Each value represents the mean of at least three separate determinations.

zyme. When 1,2-diolein was omitted from the reaction mixture, the PKC activity of undifferentiated HL-60 cells was not significantly different from that of their differentiated counterparts (**Fig. 2**). However, when 1,2-diolein was present, HL-60 cells that had been induced to differentiate by TPA, DMSO, or GM3 showed a pronounced increase in PKC activity of from 2- to 3-fold (Fig. 2). The PKC activities of HL-60 cells treated with GM1, BBG, or sulfatides were about 1.6-, 1.4-, and 1.2-fold higher, respectively, than that of untreated cells; these elevations were considerably less than those of the differentiated leukemia cells.

The activity of ST1 was measured in sonicates of HL-60 cells; incorporation of [^{14}C]NeuAc from CMP-[^{14}C]NeuAc into the lipid accepter LacCer was linear with concentrations of protein from 0.1 to 0.8 mg. Treatment with the various agents under study caused an increase in the activity of ST1, except for GM3 (**Fig. 3**). The order of the stimulatory efficacy was TPA > DMSO > sulfatides > GM1.

To gain information on the inability of GM3 to increase the activity of ST1 prepared from HL-60 cells treated with this agent, gangliosides were isolated from cells and the level of GM3 was ascertained (**Fig. 4**). The GM3 concentration in HL-60 cells treated with exogenous GM3 was much greater than that present in leukemia cells treated with the other agents. Its concentration was similar to that in cells treated with TPA (Fig. 4). The effect of added exogenous GM3 on the activity of ST1 was also examined. As shown in **Fig. 5**, the activity of ST1 declined sharply at concentrations of GM3 greater than 5 nmol in the reaction mixture, suggesting that the relatively high concentration of GM3 present in HL-60 cells treated with this ganglioside might well inhibit ST1 activity.

DISCUSSION

TPA, DMSO, and the gangliosides GM3, GM1, and BBG were shown to be potent inhibitors of the growth of HL-60 leukemia cells; some of these agents (i.e., TPA, DMSO, and GM3) are also known to induce the differentiation of these cells and this action was confirmed in the

TABLE 1. The induction of the differentiation of HL-60 leukemia cells by DMSO and TPA as measured by phagocytic activity

	Inducers											
	None			DMSO			None			TPA		
	3 d	6 d	8 d	3 d	6 d	8 d	12 hr	24 hr	36 hr	12 hr	24 hr	36 hr
Adherent cells												
(%)										12.3	48.9	55.6
± SEM										2.3	7.8	9.4
Phagocytosing cells												
(%)	3.7	4.1	10.8	46	54	55	5.1	4.7	5.8	55	66	69
± SEM	1.1	0.8	0.7	1.4	0.8	4.5	0.7	3.6	2.8	9.5	5.4	6.0
NBT-positive cells												
(%)	4.8	6.3	7.8	37	56	59	4.8	4.8	6.5	5.3	6.5	11
± SEM	0.7	1.3	1.3	5.3	2.9	5.8	0.2	1.0	0.9	0.9	1.9	3.3

The number of adherent cells was determined as described in the Materials and Methods. The percentage of phagocytosing and NBT positive cells was ascertained for at least 200 cells using a hemocytometer. Each value represents the mean ± SEM of at least three separate determinations.

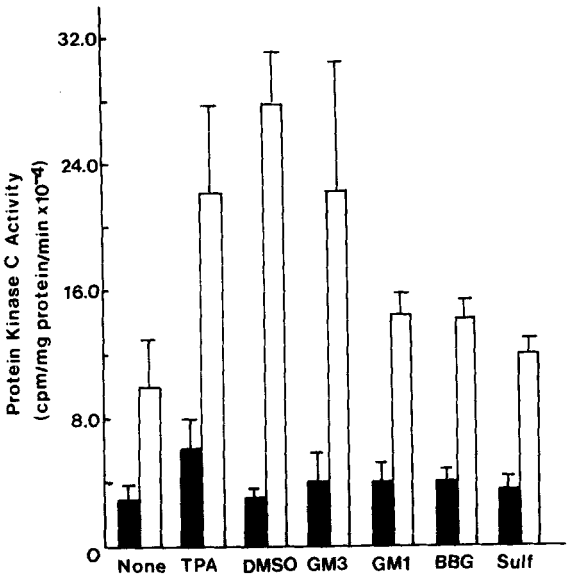

Fig. 2. Effect of various agents on diolein-stimulated protein kinase C activity. Partially purified protein kinase C was assayed in HL-60 leukemia cells treated for 3 days with either 10 nM TPA, 1.3% DMSO, 100 μM GM3, 100 μM GM1, 100 μM BBG, or 100 μM sulfatides using 200 μg/ml histone III-S as the substrate. The free Ca^{2+} concentration was 1.1 μM. Solid bars, 20 μg/ml of phosphatidylserine; open bar, 2 μg/ml of 1,2-diolein and 20 μg/ml of phosphatidylserine. The data represent the mean ± SEM. The number of determinations varied from 9 to 19.

present study. Thus, HL-60 cells treated with TPA and GM3 matured along the monocyte/macrophage lineage as had been observed previously (2, 3, 29), while DMSO induced the differentiation of these cells into granulocytes (1). Although it has been reported that ganglioside GM1 or BBG had no effect on cellular differentiation, producing instead a stimulation of the replication of these cells (4, 29), we have found that these agents actually inhibit the growth of HL-60 cells over a wide range of concentrations. The reason for this discrepancy is obscure at present. However, our results are consistent with the observations of Bremer et al. (30, 31), who reported that GM1 and GM3 inhibited cellular proliferation, inhibited platelet-derived growth factor (PDGF) and epidermal growth factor (EGF)-dependent DNA synthesis, altered growth factor binding, and reduced growth factor-stimulated protein phosphorylation. These phenomena were also observed in Swiss 3T3 cells undergoing oncogenic transformation (32–35).

Although phorbol esters have been reported to have mixed effects on the PKC activity of intact cells (36, 37), we found that TPA and other agents tested in the present study were capable of enhancing the 1,2-diolein-stimulated PKC activity of HL-60 cells. Since TPA, DMSO, and GM3, which induce cellular differentiation in this system, caused a two- to threefold increase in PKC activity, whereas GM1, BBG, and sulfatides, which did not induce maturation, elicited a smaller enhancement of PKC activity, it is conceivable that the capacity of TPA, DMSO, and GM3 to induce cellular differentiation is related at least in part to the magnitude of the enhancement of PKC activity. The mechanism by which PKC activity is increased by these agents may be due to several factors. First, DMSO, TPA, gangliosides, and sulfatides can be incorporated into the plasma membrane; such incorporation may produce a conformational change that leads to an increase in the affinity of the enzyme for Ca^{2+} and phosphatidylserine. Second, TPA, DMSO, and GM3 induce the differentiation of HL-60 cells, and the PKC activity of differentiated cells may be constitutively higher than that of their undifferentiated counterparts. Third, these agents may cause a change in the distribution of the enzymic activity between the cytosol and the plasma membrane which affects PKC activity.

We have also shown that the induced cellular differentiation produced by the tumor promotor TPA is accompanied by an increase in ST1 activity; similar results have also been reported by Momoi et al. (10) in HL-60 cells and by Moskal et al. (11) in the virally transformed clonal hamster cell line,

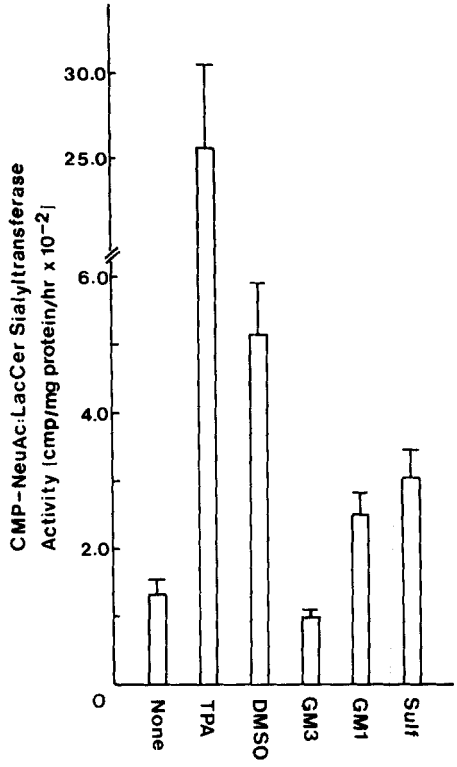

Fig. 3. Effect of various agents on CPM-N-acetylneuraminic acid:lactosylceramide sialyltransferase activity. The enzyme activity from HL-60 leukemia cells treated for 3 days with either 10 nm TPA, 1.3% DMSO, 100 μM GM3, 100 μM GM1, or 100 μM sulfatides was assayed as described in Materials and Methods. Each value represents the means ± SEM of at least three separate experiments.

NIL 8-HSV. Interestingly, the latter authors could not detect any effect on glycosyltransferases other than ST1 for glycolipid synthesis. In addition, we have also found that DMSO produces effects similar to those of TPA. These observations are consistent with the concept that the increase in GM3 synthesis is directly correlated with the activation of PKC.

In contrast, GM3 did not enhance the sialyltransferase activity of these cells. It is possible that the GM3 in the medium is inserted into the cellular membrane (38–40) as indicated in the present study, and that the residual GM3 decreased the activity of the sialyltransferase by end-product inhibition. This type of end-product inhibition has been previously shown by Yu et al. (41) and Nores and Caputto (42). This possibility is supported by our experiments in which the addition of exogenous GM3 to the enzymatic system produced inhibition of sialyltransferase activity. Furthermore, GM1 and sulfatides did not inhibit sialyltransferase activity, showing instead a slight activation of the enzyme.

The findings have led us to hypothesize that the PKC activity and the increase in GM3 synthesis may be related events that are important to HL-60 cellular differentiation. It is possible that PKC may participate in regulating the activity of the sialyltransferase by a phosphorylation–dephosphorylation mechanism. In such a mechanism, the phosphorylated sialyltransferase may be envisioned to represent the active form of the enzyme, whereas the dephosphorylated form would represent the inactive catalyst. In support of such a concept, several lines of evidence have suggested that glycosyltransferases such as ST1 (11, 43) and UDP-N-acetylgalactosaminyltransferase (44) may be regulated by protein kinase systems. Further studies are being conducted to ascertain whether phosphorylated and non-phosphorylated forms of the sialyltransferase exist in HL-60 leukemia cells.

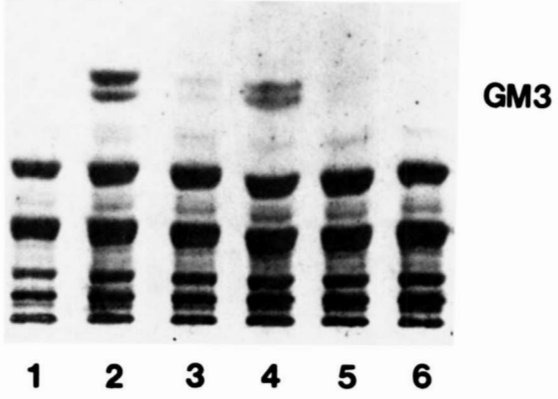

Fig. 4. HPTLC chromatogram of gangliosides isolated from HL-60 cells treated with various agents. Lanes 1, control; 2, TPA; 3, DMSO; 4, GM3; 5, GM1; and 6, sulfatides. Each lane contained the total gangliosides from cells (equivalent to 1 mg of protein) with 50 μg BBG added as a carrier. The developing solvent system was chloroform–methanol–water 50:45:10 (v/v) containing 0.02% $CaCl_2 \cdot 2H_2O$. Gangliosides were revealed by resorcinol–HCl reagent.

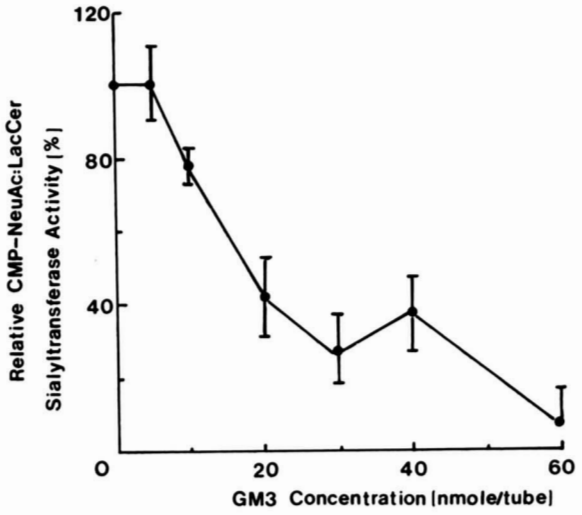

Fig. 5. Inhibition of CMP-N-acetylneuraminic acid:lactosylceramide sialyltransferase by GM3. Sonicates of HL-60 leukemia cells treated with 10 nM TPA were used as the enzyme source. The reaction mixture contained 40 nmol of LacCer, 80 nmol of CMP-[^{14}C]NeuAc, 300 μg of Triton CF-54, 2 μmol of $MnCl_2$, 500 nmol of cacodylate buffer (pH 6.3), 500 μg of protein, and various amounts of GM3. Each point represents the mean ± SEM of four separate experiments.

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